ProCipitate™

Superior Substitute to Phenol/Chloroform for DNA Isolation & Protein Binding

Handbook of applications, strategies, and potential new directions

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Introduction

There are many DNA, RNA, and nucleic acid preparation products that serve the market well for most applications. Most of these products in some way utilize a bind/wash/elute strategy adopting surface reagents such as silica & metallic oxides and similar solid-phase chemistries. However, there still remain situations when these products are not optimal. This Handbook serves to guide users in the use of **ProCipitate™** when the "other kits don't fit".

The **ProCipitate™** strategy is opposite to common prep strategies, as instead of binding nucleic acids, the protein is efficiently depleted with no interaction with the soluble nucleic acids. **ProCipitate™** is offered in a variety of kits, providing all necessary buffers and accessories for different applications: **ProPrep™ Genomic 96/100** for high throughput whole blood DNA; **ProPrep™ BAC Mini** for large insert plasmid preps.

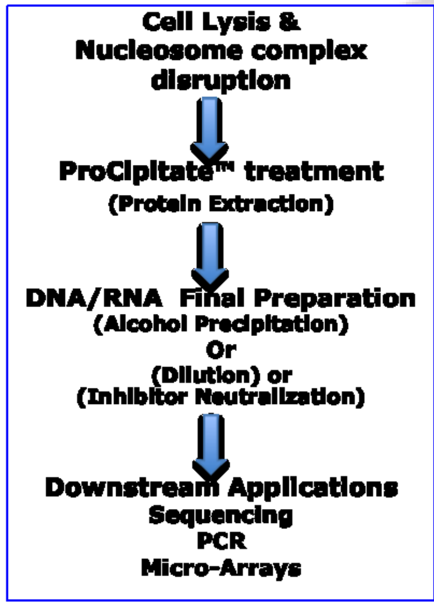
ProCipitate™ is a unique protein binding reagent developed from patented solid-phase polyelectrolytes. These elastomeric polymer suspension reagents are prepared in an extended state due to strong electrostatic repulsion of the repeating polymeric acid groups. Upon interaction with proteins, salt bridges form and the resultant complex collapses to a lower energy state, expelling water much like dehydration processes taking place within solvent precipitations. Consequently, aggregation of proteins is strongly promoted and occurs even in high ionic strength or surfactant containing solutions. Most importantly, nucleic acids remain unreacted and are quantitatively recovered in solution.

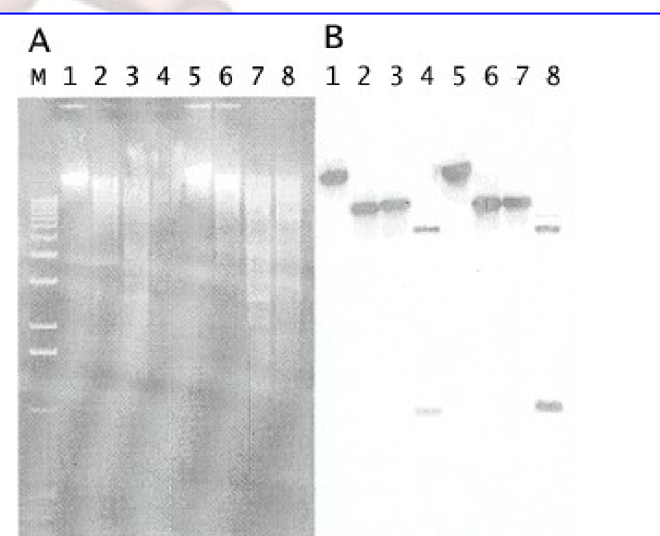
In this way, **ProCipitate™** is characteristic of phenol/chloroform separation, a long established benchmark for nucleic acid isolation. However, **ProCipitate™** is non-volatile, non-hazardous, and has the additional benefits of solid-phase suspensions; that is - the adaptability to filtration and automation.

ProCipitate™ and related kit products have been on the market for close to 20 years being used throughout the Human Genome Sequencing Project. It is routinely used and cited in protocols for improving the yield consistency and protein depleted quality of DNA. Such improvements have been cited in sequence and PCR quality for a variety of applications, most notably in the template preparation of large insert plasmids (cosmids & BACs) and PCR suitability for infectious agents from large volumes and from paraffin-embedded tissues.

ProCipitate™ can even be used for enrichment of other macromolecules including viruses, proteoglycans, polysaccharides, glycolipids, and highly substituted polymer conjugates (i.e., PEG), which serve to mask salt bridge formation and retain solubility. A full list of references is provided at the end of this Handbook.

Considerations for optimal use





Agarose gel electrophoresis and Southern blot analysis comparing genomic DNA from yeast purified by phenol/chloroform and by ProCipitate™.

A. Lanes 1-4 are isolated by traditional phenol/chloroform methods.
Lanes 5-8 were purified using non-hazardous ProCipitate™
Lanes 1 & 5 are undigested.
The other lanes were digested with restriction enzymes as follows:
(BAM HI – lanes 2 & 4),(Eco RI – lanes 3 & 7), (Hind III – lanes 4 & 8)

B. Southern Blot. The DNA was transferred and hybridized to a labeled probe and exposed to film for 3 hours.
Lanes 1-8 correspond to A.

The optimal use of **ProCipitate™** for nucleic acid isolation should consider these 4 necessary processes:

1. The cells/tissue must be sufficiently lysed so that the intracellular fraction is released into the surrounding media, and the nucleosome (histone protein/DNA complex) is efficiently disrupted.

2. The amount of **ProCipitate™** required will depend on the starting sample protein load, guidelines for which are provided further on in this Handbook, and

3. The soluble DNA upon treatment, must be finally prepared to neutralize any inhibitory effects of the lysis condition. Typically, this is done by alcohol precipitation, dilution, or chemical neutralization; for which some of these protocols are documentted in the references provided. Because **ProCipitate™** is reactive under diverse lysis conditions, the user has great latitude in designing a protocol optimized for their own particular application.

4. **ProCipitate™** reactivity is indistinguishable between DNA and RNA.

Performance Characteristics

Sample Size	ProCipitate™ Typical Usage
1 ml Yeast Culture Genomic DNA	200 µl
Mouse Tail Genomic DNA	200 µl
1 mm Plant Leaf	50 µl
2.0 ml culture BAC Preps	80 µl
5µm paraffin-embedded tissue	200 µl
250 µl culture Plasmid Preps	20 µl
200 ml Large Scale BAC Preps	5 ml
Dried Blood Card or ~ 40 µl Whole Blood	200 µl
200µl lysed cell pellet	200 µl

Protein	ProCipitate™: Sample	Removal
BSA, PBS @ 30 mg/ml	1 : 1	>99%
BSA, 1%SDS @ 30 mg/ml	1 : 1	>99%
BSA, 3M GuSCN @ 30 mg/ml	1 : 1	>99%
Human Serum	1 : 1	>90%
Human Serum, 1% Surfactant	1 : 1	>95%
Nucleic Acid Recovery	**ProCipitate™: Sample**	**Recovery**
Calf Thymus DNA, A_{260} = 1.00	1 : 1	>95%
Total RNA, A_{260} = 1.00	1 : 1	>99%

ProCipitate™ performs optimally in a final pH range of approximately 4 to 6, however the polyelectrolyte is sufficiently acidic (pH 4) to lower the final reaction pH to within its optimal working range in most applications. Detailed protocols for use can be found at:

http://biotechsupportgroup.net/productsheets/Procipitate%20Product%20Sheet%20111715.pdf

For Protein Enrichment Applications
For applications involving enrichment of macromolecules other than nucleic acids, the same general protocols can be used. For extracellular macromolecules, no additional lysis considerations are necessary.

ProCipitate™ appears in several articles and books on Food Safety

- **Foodborne Disease Handbook,** Second Edition,: Volume 2: Viruses: Parasites
  By Y. H. Hui, Sayed A. Sattar, Wai-Kit Nip
  "Viruses in the PEG eluants were precipitated…by an equal volume of ProCipitate™."

- **Health-related Water Microbiology,** Volume 27, Issues 3-4, Pergamon, 1993
  "ProCipitate™ was an effective method to purify the sample and dramatically improve virus detectability by RT-PCR."

ProCipitate™ is Scaleable

The volumetric ratio of ProCipitate™ to sample can be adjusted up or down depending on the concentration of protein in the sample. Once established, these same ratios can be used to process volumes at any scale.

ProCipitate™ is also packaged within application specific kits – called **ProPrep™**, which include the lysis and resolubilization buffers. These include:
ProPrep™ Genomic 96/100 for whole blood DNA, & **ProPrep™ BAC Mini** for large plasmid preps.

ProPrep™ Genomic 96/100

Whole Blood DNA Purification System with ProCipitate™

ProPrep™ Genomic 96/100 is a complete nucleic acid purification system based upon the unique protein extraction reagent, **ProCipitate™**. The basic protocol includes one step chaotrope lysis of 50 µl whole blood (no buffy coat reduction), followed by removal of contaminating proteins and heme with **ProCipitate™**.

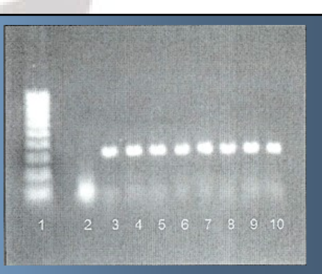
The **ProPrep™ Genomic 96/100** permits the user to customize a massive PCR, SNP or NGS strategy without regard to collecting impractical quantities of whole blood. The isolated DNA is of the highest quality, and PCR can be achieved from as little as 1 ng of template DNA. This means that over 1,000 PCR reactions can be obtained from one, 50 µl whole blood sample.

Options – After separation, the purified DNA is contained within the lysis buffer. The DNA can then be either alcohol precipitated or simply diluted using a "Dilution Protocol", to eliminate inhibitory effects of the lysis buffer, see results below. Note- protocols for separating the polymer-protein composite can either be done by centrifugation or filtration. 96-well plates are not stocked but recommendations for suitable suppliers can be made. The **ProPrep™ Genomic 96/100** protocol describes a 96-well filtration process that can be completed in 30 minutes or less.

Dilution and PCR – No alcohol precipitation

The volume recovered after filtration was approximately 250 µl. A 1:50 dilution was made and PCR was performed from 10 µl aliquots. Thus the number of projected PCR reactions from the final volume is 1,250. 50 µl of whole blood contained ≈ 1,270 ng DNA; final diluted volume = 12,500 µl, contained 1,270 ng DNA or 0.1 ng/µl; 10 µl or ≈ 1 ng was used as input to PCR. For detailed protocol, visit:

Lane1: 100-1000 bp ladder
Lane 2: Negative control
Lanes 3-10: PCR amplicaons from **1 ng** template DNA purified from whole blood, randomly selected from 96 wells.

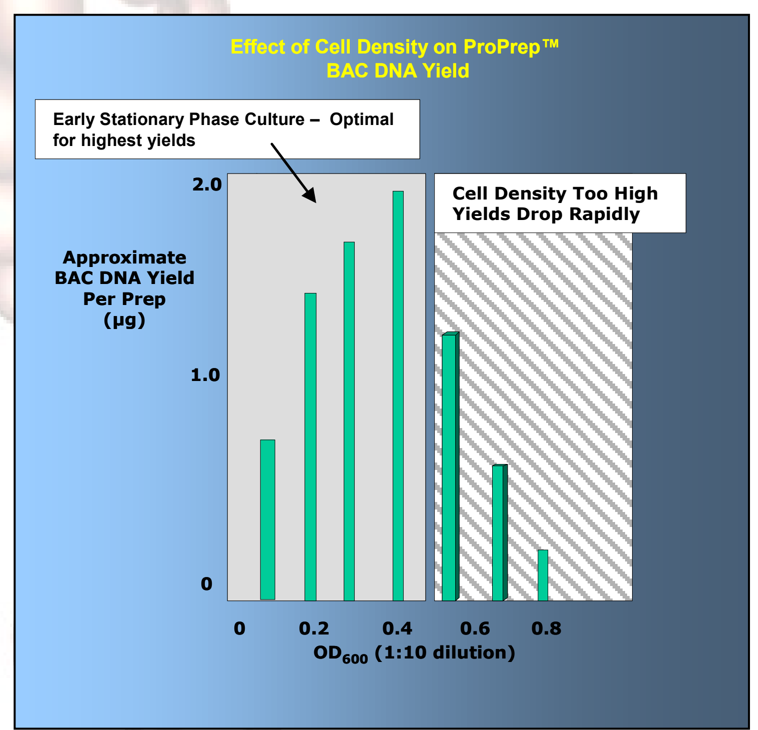


Amplicons are 280 bp from Human HLA-DR-Beta primers at 32 cycles.

ProPrep™ BAC Mini

Mini-Prep for BACs & Large Plasmids Using ProCipitate™

ProPrep™ BAC Mini is a complete microfuge based purification system based upon the proprietary reagent, **ProCipitate™**.



ProCipitate™, used throughout the Human Sequencing Project for BAC isolations, has been demonstrated to provide high quality DNA suitable for automated fluorescent sequencing of small to large insert DNA.

ProPrep™ BAC Mini starts with 2ml overnight cultures, and then utilizes ProCipitate™ in a modified alkaline lysis protocol. While this kit is adaptable to 96-well plates, plastic components are not stocked but recommendations for suitable suppliers can be made.

Product Ordering Information

ProCipitate™ &
Superior Substitute to Phenol/Chloroform for DNA Isolation & Protein Binding

- Removes protein contaminants & leaves DNA soluble and unreacted
- Ideal for applications when the alternative kits don't fit, or are not optimal
- Adaptable to any sample size and can be automated
- Pathogen and infectious disease testing
- Tissue and paraffin-embedded tissues

Product	Size	Item No.
ProCipitate™	30 ml	P0050-30
ProCipitate™	100 ml	P0050-100

Accessory Item	# Spin-filters	Item No.
Corning Costar Spin-X ® 0.45 µm Cellulose Acetate 0.5 ml Filter, with 2.0 ml tube	25	SPX-1

ProCipitate™ and related products can be customized to fit specific needs. It also can be supplied in bulk quantities. Please contact our sales office or any of our worldwide distributors for more information.

ProPrep™ Genomic 96/100

Whole Blood (50 µl) DNA Purification System with ProCipitate™

- PCR can be achieved from as little as 1 ng of template DNA
- Does not require reduction to buffy coat, filtration compatible, no precipitation
- Customize a massive PCR, SNP or NGS strategy

Product	Size	Item No.
ProPrep™ Genomic 96/100	96-100 preps	PPG-100

ProPrep™ BAC Mini

Mini-Prep for BACs & Large Plasmids Using ProCipitate™

- Ideal for mini-prep BAC template preparation from 2.0 ml 2xYT cultures.
- Adaptable to 96 well and automation.
- Protocols served the Human Genome Sequencing Project

Product	Size	Item No.
ProPrep™ BAC Mini	96-100 preps	PMK-100

References

Patents

Composition and utility patents for ProCipitate™ and related technologies are covered under U.S. Patents Numbers 5,294,681, 5,453,493 & 5,658,779.

U.S. Patent Number 5,538,870, *Method for Preparing Nucleic Acids For Analysis And Kits Useful Therefore*. This patent shows the beneficial effects of ProCipitate™ in protocols which neutralize SDS with non-ionic detergents, are PCR compatible, and require no alcohol precipitation.

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- Its products are trusted brands with 125+ references, having been supported in peer reviewed journals, patents, and technical posters
- It is a leader in research consumables and products for hemoglobin, albumin, lipid, glycoprotein, viral genomics and proteomics sample preparation and enrichment.
- Its products are for research use only.

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